Home Office

NON-TECHNICAL SUMMARY

Investigation of the factors involved in the development of myeloid malignancies, and ways to prevent and treat them

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

blood, cancer, inflammation, prevention, therapy

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

In this project, we want to understand how a group of blood cancers called myeloid malignancies, start and grow. These cancers happen more often in older people, but we don’t know why. Our project focuses on:

1. Learning how these cancers begin and develop.
2. Figuring out why they are more common in older people.
3. Finding better ways to treat or even prevent them.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our blood system keeps us alive by doing things like carrying oxygen, fighting germs, and helping heal injuries. But when it doesn’t work properly, it can lead to serious illnesses, including life-threatening blood cancers like myeloid malignancies. These cancers become more common as people get older, affecting more than 15 in 100,000 people every year. As the world’s population ages, this number will increase, making it a bigger health problem. Unfortunately, current treatments aren’t very effective, and most patients have a poor quality of life and still die from these diseases. This is because we don’t fully understand how these cancers start or why they get worse over time. Our goal is to uncover the causes of these cancers, which will help us create better treatments. We also want to prevent these cancers from happening in the first place. In the past, prevention wasn’t possible because people often got sick suddenly, without warning signs. But recent research, including our own, has shown that it’s now possible to identify people at high risk of developing these cancers years before they get sick. This gives us a chance to find ways to stop the disease before it starts.

These cancers begin in blood stem cells. Blood stem cells are responsible for producing all the blood and immune cells we need throughout our lives. Over time, these stem cells can get damaged and develop mutations (alterations in their genetic material). Most of the time, these mutations don’t change how the cells work. But sometimes, the mutations give the stem cells an advantage, allowing them to grow faster and outnumber normal cells. This condition is called clonal haematopoiesis (CH). CH becomes more common as people age, affecting over 30% of people aged 70 and older. While most people with CH won’t develop cancer, they do have a higher risk.

Downloaded: 8:42am, 26 Sep 2025

Apart from mutations, other factors can affect how blood stem cells behave. Things like aging, infections, inflammation, diet, alcohol, smoking, exercise, sleep, and even the microorganisms living in our bodies all play a role, and for some mutations, age has a particularly strong effect. These mutations may seem harmless in younger people, but they can lead to cancer in older people. However, we don't yet understand how these factors interact with the mutations to cause cancer. One of the aims of this project is to study these interactions. By learning more about what triggers the growth of mutant stem cells, we hope to find ways to stop the process and prevent cancer.

What outputs do you think you will see at the end of this project?

This project aims to learn more about what causes myeloid malignancies (a group of blood cancers) to start and get worse. With this new knowledge, we will create tools in the laboratory and on computers to identify people who might be at high risk of getting these cancers and predict how their disease will develop and progress. We also want to discover new medicines or test whether existing medicines that are used to treat other diseases could help to prevent or treat these blood cancers. Our ultimate goal is to help patients live longer and have a better quality of life.

We will share what we learn with other scientists by giving talks at conferences and writing about our findings in articles for publication.

Who or what will benefit from these outputs, and how?

In the short-term, our research will help doctors, scientists, and pharmaceutical companies by providing them with new ways to detect, prevent and treat myeloid malignancies.

To help patients quickly, we will work with other scientists, medical doctors and biomedical companies to turn our findings into new tests and treatments. We have experiences with this process and are already making progress with drug trials based on similar studies.

In the longer term, our work will lead to better ways to prevent and treat myeloid malignancies and similar blood cancers. This will mean that patients can live longer and have a better quality of life, which will also help their families. Overall, preventing and improving treatment for these cancers will be good for our society in many ways.

We enjoy sharing our research with the public and will continue to do so to help people understand our work. This will also match the public engagement goals of our institution.

How will you look to maximise the outputs of this work?

We will share our findings with other scientists by publishing our results in scientific journals and presenting them at scientific conferences and meetings. The datasets we generate will be stored and can be accessed by other researchers around the world via the European Nucleotide Archive (ENA) at EMBL-EBI.

New animal models we create, and the cells collected from these mice will be helpful for other scientists studying myeloid malignancies or related inflammatory diseases like heart conditions. We will freely share our mice and research tools with non-commercial organisations after our findings are

Downloaded: 8:42am, 26 Sep 2025

published. We will also allow other scientists to use our models before publication for collaborative studies. We have already shared many of our mouse models with other universities and research centres around the world. Additionally, we plan to share and use tissues from aged mice through SHARM (https://www.sharmuk.org).

Species and numbers of animals expected to be used

- Mice: 40100

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are commonly used in biomedical research because they are similar to humans in many ways, including their body structure, how their organs work, and their genetic makeup. They are also easy to care for in a laboratory environment. New technologies have made it possible to efficiently create mouse models that are very useful for studying myeloid malignancies. These mouse models have specific genetic changes (mutations) that are like those found in patients with myeloid malignancies.

Some mutations linked to myeloid malignancies can be found even before birth, but these mutant cells grow much more in older individuals. Therefore, understanding how these mutations affect the blood system in early life and how factors associated with ageing drive the dramatic growth of mutant cells and changes in their bone marrow environment is a key part of our project. Mice are especially useful for this research because their blood system and ageing process are similar to those of humans. Since mice live about 125 weeks, we can study these processes and complete our research in a reasonable amount of time.

Because mice are widely used in research, there are many well-established methods for studying their blood and bone marrow. One common method is called flow cytometry, which allows scientists to identify or separate specific cells. This is done by using special fluorescent markers that attach to the cells of interest, making them easy to study further.

Typically, what will be done to an animal used in your project?

Most of the methods and procedures needed for this project are well-established in blood cancer research and within our group. We have successfully used them in the past to study different mouse models of myeloid pre-malignancy and malignancy.

In practice, we will breed mice to produce offspring with specific genetic alterations. To activate these genetic changes, we sometimes use a procedure that involves one or more intraperitoneal ((i. p.), into the peritoneal cavity) injections of a substances to promote turn the genetic alteration on or off. Some of these experimental mice may live up to 125 weeks after genetic activation. During this time, we will

Downloaded: 8:42am, 26 Sep 2025

collect blood from the mice from tail vein or saphenous veins (located in the legs) at various intervals to monitor how the number of blood cells changes as a marker of disease progression in mice carrying one or several genetic alterations or via cardiac puncture if analysis of a large volume of blood is absolutely required. This is a bleeding method by collecting blood directly from heart and, a terminal procedure that is conducted under deep anaesthesia and animal will then be humanely killed.

To investigate how factors that mimic the real-life human exposures such as infections, inflammation, diet, aging-related changes, toxins, and other external or internal influences, dysregulate blood stem cells and causes myeloid malignancies, we will treat some experimental mice with substances that cause acute infection (for example, one dose of intraperitoneal injection of poly(I:C), chronic infection ((i.e., multiple doses of intraperitoneal injection of poly(I:C) or bacterial cell elements such as LPS)), low-grade inflammation (high-fat diet or poor diet choices) or substances that cause abnormal activation or inactivation of proteins that are associated with myeloid malignancies or substances that mimic ageing related factors. These treatments may last up to 12 weeks and are expected to cause long-lasting changes in stem cell properties, but the extent of these changes may be subtle, similar to those observed in humans exposed to these factors. We will also study the effect of anti-inflammatory treatments with various substances to modify the behaviour of the altered blood cells or their environment. The treatment may involve single or multiple administrations of a substance through different delivery routes (e.g. in food, drinking water, via subcutaneous or intraperitoneal injection). The frequency and volume of these treatments are not expected to harm the animals long-term. Additionally, mice may undergo behavioural modifications, such as changes to their light-dark cycle, a modified diet, or exercise equipment such as treadmills, rotarods, and grip meters, to indirectly influence blood cell activity.

Transplantation will be performed only once in a mouse's lifetime. Transplantation involves injecting donor cells into recipient mice either via a non-invasive route, such as tail vein injection, or into the marrow cavity of a bone like the femur under anaesthesia). Before receiving a donor cell transplant from another mouse, some recipient mice will undergo irradiation to weaken or remove their own blood system, so the host bone marrow cells do not compete with the donor cells for space, allowing donor cells to efficiently engraft (settle in the host bone marrow) and produce blood and immune cells. Depending on the amount of irradiation, mice may experience post-irradiation illness, but our advanced care plan will effectively manage this condition. When relevant, human blood cells may be transplanted into mice to confirm findings from mouse studies. The transplanted cells may also be pre-manipulated in vitro (outside the body, usually in a petri-dish used in laboratories) to enhance their potential to generate leukaemia in mice. Occasionally, pre-manipulated cells may emit bioluminescent light, enabling imaging of cell growth within the body. Upon injection of an imaging substance into the mice, this light can be captured from living mice placed in an imaging system. To ensure stress-free restraint during imaging, mice will undergo short-term anaesthesia and are expected to recover without long-term harm.

Anti-leukaemic treatments may be administered to prevent, slow down, or eliminate leukaemic cells. These treatments may involve repetitive handling of mice for brief moments to administer the substance by injection. The frequency and volume of these treatments are not expected to harm the animals long-term.

As myeloid malignancies-linked mutations are sometimes found before birth, to understand how they affect the blood system in early life, sometimes we may inject manipulated cells (such as embryonic

Downloaded: 8:42am, 26 Sep 2025

stem cells edited in a petri dish) into early-stage embryos to study how the manipulated cells affect early-stage blood system formation.

In some cases, a large volume of blood may be required for analysis. To obtain this, a mouse may be placed under terminal anaesthesia (a state of deep sleep followed by euthanasia) to collect a final blood sample from the heart. Similarly, terminal anaesthesia may be used to perfuse the animal with a fixative solution to preserve its organs for further analysis.

At the end of experiments, mice will be humanely killed. After euthanasia, we will collect different organs (e.g. bone marrow, spleen, liver) to analyse multiple parameters such as the proportions of different cell types, gene expression, and protein expression.

What are the expected impacts and/or adverse effects for the animals during your project?

Trained personnel in the animal facility and our research team perform regular health monitoring for all mice. With our extensive experience in studying mouse models of blood cancers, we understand the potential adverse effects of our experimental designs and can enhance care for mice at increased risk of ill-health. Any mice showing signs of ill-health or significantly reduced activity will be humanely killed within 24 hours depending on severity and the type of the clinical signs. Additionally, when visible signs of compromised well-being (piloerection characterised by the body fur standing on end, hunching, reduced activity for more than 2 hours) are observed, enhanced monitoring and support will be implemented according to the care plans included in this license.

During our project we will use different procedures and experimental setups that are expected to have varying levels of impact on animal well-being as listed below.

1. Breeding of Genetically Altered Mice:

Breeding genetically altered mice is not expected to cause harmful effects. Most genetic changes used in our project will manifest in adult animals used for experiments, so the majority of breeding animals will not experience adverse effects from changes in their blood system.

2. Induction Treatments:

To activate genetic alterations, substances will be injected into mice up to seven times, either daily or every other day. Mild weight loss of up to 15% may occur due to the biological effects of the injected substances and routes of administration. When tamoxifen is administered via injection under the skin, abdominal swelling and scrotal swelling may be seen. These adverse effects will subside once the induction period ends and will not have long-term effects on the mice.

3. Blood Collection:

Blood samples will be collected from a superficial vessel such as the tail vein up to 20 times during a mouse's lifetime. Sample volume will not exceed 10% of total blood volume in 24 hours or 15% of total blood volume in any 28-day period tail vein bleeding. This process may cause short-term discomfort and stress but is not expected to have long-term effects on the animal.

4. Blood Cancer Development:

Downloaded: 8:42am, 26 Sep 2025

In most of the cases, genetic alterations causing the cancer will be activated in mice only upon a specific treatment. As the timing of the alteration activation is under our control, we are able to predict when the disease should begin and intervene early to minimise adverse effects.

Some mice will develop blood cancer, showing gradual changes in blood cell numbers, which may lead to an enlarged spleen or liver. Occasionally, other tumours may appear. Signs of tumour growth, such as a swollen abdomen or lumps under the skin, and advanced disease symptoms like a hunched posture, raised hair, and reduced activity, will be humane endpoints.

5. Blood Cell Transplantation:

Blood cells will be transplanted into some mice, usually via the tail vein. This procedure may cause temporary discomfort and stress but is not expected to have long-term negative effects. In specific cases, abnormal blood cells may be injected into the cavity of a thigh bone to improve transplantation outcomes or modify experimental results. This route of injection causes more pain and distress to the mice, so it will be conducted under anaesthesia and only when it is absolutely required. To ameliorate the pain 1) trained personnel will perform the procedure using aseptic techniques to minimise duration and trauma; 2) mice will be monitored for pain or distress (e.g., reduced movement, changes in grooming, hunched posture) and provided with enriched food, water and an improved physical environment; 3) recipient mice will receive pain relief medication.

In some recipient mice receiving leukaemic cells, the cells may infiltrate (get into) the area around the nerves coming out of the spine. As these nerves are responsible for movement of the back legs, this can initially cause weakness (also known as paresis) and this sometimes can progress to full paralysis. Based on previous experience, this clinical sign may occur in some recipient mice between approximately 3 to 6 weeks post transplantation (this can vary slightly between different leukaemic cells). This symptom can be distinguished from a generalised reduction in their ability to move around; specifically, the front legs are unaffected, but the back legs are weak. These signs will represent the humane endpoints, and the mice will be culled when the humane endpoint is observed.

In such experiments where recipient animals received leukaemic cells, to minimise the possibility of paralysis, drug treatment will be applied within the first week after transplantation so effects of new therapies could be observed before 3-6 weeks when paralysis may happen. To reduce animal suffering, from third week after transplantation we will include additional monitoring from the third week to catch any early sign as this is the period when animals would be more susceptible to this happening. In addition, in case this occurs, and animals cannot rear up to eat/drink, we will include floor food/wet mash/diet gel on the cage floor from week 3, so animals are not starved/dehydrated before they are found unwell, and the humane endpoint is applied.

6. Preconditioning for Transplantation:

Mice may undergo irradiation or other methods before transplantation. Irradiation is a standard method to prepare mice before transplantation. Following irradiation, the mice suffer from post-irradiation illness, where decreased food and water intake are main adverse effects and may lead to weight drop. The severity depends on the dose; low doses may result in mild weight loss (below 10%) and unchanged activity, with mice recovering within 7-10 days whereas high doses can cause more pronounced illness and weight loss of up to 15%. Recovery is also expected within 7-10 days. Partial body irradiation may require 20-30 minutes of anaesthesia but will not have long-term adverse effects.

Downloaded: 8:42am, 26 Sep 2025

Mice will be weighed before irradiation and every other day to detect weight drop as a sign of ill-health post transplantation. Weighing of mice will continue until the weights are stabilised. To prevent the post-irradiation illness and weight drop, mice may be given enriched diet (mashed food, and/or Dietgel) that may continue for at least 5 days post-irradiation. If weight loss exceeds the anticipated limit, mice will be provided with an enriched diet until their weight become stable.

Preconditioning will be done only once in a mouse's lifetime.

7. Surgeries:

Surgeries such as splenectomy (i.e., removal of spleen, a method sometime used in clinic to alleviate the symptoms of leukaemia patients), will be performed under anaesthesia in germ-free conditions to prevent infection. Post-surgery, mice will be kept warm until fully recovered. Pain relief will be administered, and mice are expected to be fully active within two hours after surgery. Any signs of prolonged inactivity or pain will be humane endpoints.

8. Imaging:

Mice may undergo non-invasive imaging sessions lasting up to 45 minutes, with a maximum of nine sessions. Each session involves anaesthesia, and mice are expected to recover without long-term effects.

9. Anti- or Pro-Disease Treatments:

Treatments for up to 16 weeks to alter blood cancer progression will involve substances with known moderate adverse effects, such as reduced activity, decreased feeding, and weight loss of up to 15%, which may persist throughout the treatment period.

10. Physiological Treatments:

Physiological treatments, such as high-fat or nutrient-deficient diets, exercise, inflammation, infections, changes in gut bacteria, and light-dark cycle disruption, may result in mild changes in activity and weight, up to 10% of pre-treatment weight. These treatments will last up to 12 weeks.

11. Aging related processes:

Aging as a physiological process may cause mild effects like reduced activity, weight changes, hair loss, or blindness. Aged mice will benefit from enhanced care and will be euthanized if they show signs of suffering. In rare cases, older mice may develop cancers or experience faster disease progression.

We will mimic ageing-related conditions through multiple approaches, including inducing mild inflammation, infection, disturbed dark-light cycle, or dietary changes. These treatments may lead to weight drop as an adverse effect. The weights of mice will be checked at least twice weekly. To counteract the weight loss, mice will receive enriched diet before and throughout the treatment period to maintain their food intake.

All work will be conducted according to the principles set out in the Guidelines for the Welfare and Use of Animals in Cancer Research.

Downloaded: 8:42am, 26 Sep 2025

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect a mild severity in the majority of mice ~76% and estimated moderate severity in 24% of the mice.

What will happen to animals used in this project?

- Kept alive at a licensed establishment for non-regulated purposes or possible reuse
- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Human and mouse blood production processes and aging are physiologically similar, allowing us to use findings from mouse studies to understand human biology and validate research. The regulation of the blood system, especially the control of blood stem cells and their descendants, is not fully understood. Recreating the complex interactions between blood cells and their regulatory factors in a laboratory setting is challenging. Therefore, studying these processes in a living organism like mice provides a complete framework necessary for understanding both normal and diseased blood systems.

Technological advancements have made it possible to create mouse models with the same mutations found in patients with blood cancers. The mouse blood system is well-studied and provides a valuable resource for researching the development and function of normal and mutated blood cells. Differences between normal and diseased cells can be subtle, and animal studies can offer more precise testing conditions.

Mouse studies have a proven track record in advancing both basic and translational cancer research. Our work is increasingly focused on developing treatments, and it is crucial to test potential therapies in living animals before determining their suitability for humans.

Which non-animal alternatives did you consider for use in this project?

In our project, we use a variety of methods to answer different questions.

Most of our work is done in vitro (outside the body, in laboratory petri dish) where we grow immortalized cells (cells that do not die) as established cell lines.

Downloaded: 8:42am, 26 Sep 2025

Human samples from both healthy individuals and patients are crucial for our project. We have arrangements and tissue banks in our institute to obtain fresh and archived samples of human bone marrow, blood and blood-supporting cells. These samples are used for cultures (growing cells in petri dishes) and other experimental analyses.

Using computer-based tools (bioinformatics) we analyse newly generated data as well as existing datasets and datasets published by other researchers to interpret data from animal, human and cell line studies. This helps us answer our research questions and identify new directions for our studies. For example, these bioinformatic analyses will help identify how mutant cells behave and what they are sensitive to. These analyses are designed for establishing molecular mechanisms and analysis of cell states to determine whether they are more likely to grow or die. Ultimately, this approach has proven to be an efficient way to gain knowledge and answer some of the questions without extensive animal experiments being performed.

Why were they not suitable?

Due to the limited understanding of how the blood system is regulated in both mice and humans, it's not possible to fully recreate the natural conditions for culturing blood cells "in a dish." Once bone marrow or blood cells are removed from the body, they quickly lose their natural properties, making it difficult to study their biology accurately. Culture systems provide a simplified and artificial environment, allowing only specific questions to be studied without the full context.

In addition, the activity of drugs and the duration of their activity differ in a culture dish compared to their activity in an animal body. Therefore, studies in living animals enable scientists to draw conclusions that are more physiologically relevant. Therefore, findings from cultured cells must always be validated in living animals to confirm their significance.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We are committed to minimising the number of animals used in our research. This starts with careful planning of each experiment. We rely on results from previous experiments and statistical tools to estimate the number of animals needed to answer our research questions. It’s important to strike the right balance-using too few animals can waste resources just as much as using too many.

In our project, more than half of the animals will be used for breeding to create mice with specific genetic combinations for experiments. Some of our studies require animals to have two to four genetic changes, which can involve multiple breeding steps and result in a higher number of breeding animals. To make this process as efficient as possible, we optimise breeding strategies to obtain offsprings that

Downloaded: 8:42am, 26 Sep 2025

carry the required genetic profiles, which means that the least surplus mice will be generated. We also ensure only healthy mice are used for breeding to avoid producing animals unsuitable for experiments.

Regarding the number of experimental animals needed for answering a question, we base our estimates on experience from past studies within our group and use statistical tools (established formulas) to determine the numbers of mice needed to find significant differences between groups for each study.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We follow strict guidelines to plan and carry out our experiments.

Study Planning:

- We use the PREPARE guidelines to carefully design studies and ensure quality.
- A detailed protocol is written for each experiment, including a clear description and the number of animals needed per group.
- We optimise experimental planning to ensure that repeat procedures are avoided by collecting multiple types of data from a single procedure or sample.
- We perform pilot studies to refine methods and determine the minimum required intervention, reducing unnecessary animal use and harm.
- If needed, we consult with expert statisticians to refine our plans.

Collaboration and Transparency:

- Plans are shared with animal facility staff for smooth execution.
- Results will be published in open-access journals, following ARRIVE guidelines.
- Negative results may also be published to prevent others from unnecessarily repeating the same work.

Data Sharing:

- Genetic and sequencing data will be stored in the European Nucleotide Archive (ENA), making it accessible to researchers worldwide.

Reducing Animal Numbers:

- We use advanced methods like single-cell technologies (which allow the study of individual cells in detail rather than examining a group of cells collectively (through bulk analysis)) and bioinformatics (data analysis on computer) to get more insights from fewer animals.
- Cells from one animal are used for multiple experiments whenever possible.

Downloaded: 8:42am, 26 Sep 2025

By adopting these strategies and seeking professional advice when needed, we aim to minimise animal use while ensuring high-quality research.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Using Existing Resources:

Whenever possible, we will obtain mice with the desired genetic changes from other researchers or facilities instead of creating new ones. This helps reduce the total number of mice required. However, if suitable models are unavailable and a specific variation or genetic background is essential, we will create new models using efficient techniques.

Efficient Breeding Strategies:

We frequently require mice with a combination of two or up to four genetic modifications. To generate mice with complex genetic profiles, we employ carefully planned breeding strategies to maximize the number of offspring with the desired genetic combinations. Whenever feasible, we use homozygous mice (with two identical genes) for mutations, provided these animals are healthy and fertile, to further streamline breeding.

Cryopreservation Benefits:

We regularly review mouse colonies to maintain only necessary lines. Mice without the required combinations of genetic alterations will be killed as surplus as soon as they are examined. To avoid continuous breeding, lines not expected to be used for at least a year are cryopreserved (frozen).

- Freezing reproductive material (sperm or embryos) safeguards against facility-wide risks, such as disease outbreaks, and reduces ongoing breeding needs.

- Best practices, including cryoprotective additives, are applied to improve recovery rates, even for historically challenging strains. This approach minimizes the number of female mice required.

- Cryopreservation also preserves valuable mouse lines for future use in case of emergencies.

Collaborating and Data Sharing:

We share our mouse lines and surplus mice with other researchers to prevent duplication of effort and reduce the need for additional breeding. Additionally, we publish all results, including negative findings, to avoid unnecessary repetition of experiments.

Using Relevant Controls to Minimise Variability:

To reduce the number of animals required for reliable results, we prioritise using littermates (siblings without genetic modifications) as controls. These controls share genetic material and environmental conditions with test animals, minimizing variability. For certain studies, we use standard mice (e.g., C57BL/6) sourced from specialised facilities to ensure consistency while avoiding unnecessary in-house breeding.

Downloaded: 8:42am, 26 Sep 2025

Using Randomisation and Blinding:

To give confidence in results and reduce likelihood of repeating studies, we will use randomisation and blinding. For example, in interventional studies, we will randomise animals to treatment vs placebo and keep operators blinded wherever possible.

Maximising Data Per Mouse:

- Whenever possible, we collect multiple types of data from the same experimental animal to maximise the information obtained while minimising variability and animal use.

- We also review existing literature and collaborate with other researchers to refine experimental designs and avoid redundant studies.

Leveraging Advancing Technology:

We stay informed about new high-throughput and single-cell technologies that enhance data collection while reducing the number of animals required. These cutting-edge tools allow for high-quality, comprehensive datasets from rare cell populations, improving the efficiency and impact of our research.

Studies of Ageing related process:

Given the higher prevalence of myeloid malignancies in older populations, our studies on ageing aim to understand how ageing-related factors influence blood systems and malignancy development. We optimise the use of aged mice by collecting various types of samples and sharing unused tissues through initiatives like ShARM UK.

Balancing Efficiency and Scientific Rigor:

- While minimising animal numbers, we ensure that sufficient mice are used to produce reliable and reproducible results.

- Long-term studies, such as those focusing on ageing may require additional animals to account for natural variability over time.

By following these strategies, we aim to conduct high-quality research while minimizing the use of animals and adhering to ethical standards.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Downloaded: 8:42am, 26 Sep 2025

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

In this project, we will use mice with specific genetic changes (mutations) to study why certain blood cancers, called myeloid malignancies, develop and progress. These cancers occur when myeloid blood cells grow uncontrollably.

There are around 50 gene mutations linked to this group of cancers, and our team has already created and studied several mouse models with these mutations. We have extensive experience working with these models, allowing us to carry out experiments effectively while minimizing unnecessary harm to the animals.

In most cases, the mutation in the mice is inactive, meaning the mice remain healthy. We can activate the mutation using specific treatments, causing gradual changes in their blood system. Since we control when the mutation is turned on, we can predict when the disease will start and take early steps to reduce any harmful effects. Most of the mutations we study cause slow-progressing diseases, which helps us further limit the impact on the animals' well-being.

We will also explore how ageing-related factors affect these blood cancers by using mice with genetic changes linked to ageing. For example, we might use mice with altered immune or nervous systems or changes in metabolism. Before introducing a new model, we will gather detailed information about its specific needs and create a tailored care plan if required.

Finally, much of our research uses material collected from mice after they are humanely killed, meaning the majority of our work does not involve causing suffering to the animals.

Why can’t you use animals that are less sentient?

As detailed below, the unique biological complexity and genetic tools provided by mice are essential for achieving our study's objectives. Alternatives, such as invertebrates or lower vertebrates, lack the physiological relevance required for accurate and reproducible results.

1. Biological Relevance of the Model

The chosen species must match the biological characteristics required to answer the research question. For example:

- Mice are genetically similar to humans and have a well-characterized genome, making them indispensable for studying human diseases.

- Less sentient organisms, such as invertebrates or lower vertebrates, do not share the physiological, genetic, or cellular similarities needed for translational research.

- For complex processes like immune response, neurobiology, or cancer development, mice are the minimum viable model that accurately replicates human-like systems.

2. Availability of Genetic Tools

Downloaded: 8:42am, 26 Sep 2025

- Mice offer advanced genetic tools (e.g., transgenic and knockout technologies) that are unavailable in less sentient species. These tools allow researchers to modify specific genes and study their role in disease development and progression.

- Such precise genetic manipulation is critical for studying specific pathways and cannot be replaced by less sentient animals.

3. Physiological Complexity

- Certain studies require a vertebrate model to replicate complex organ systems, such as the cardiovascular, nervous, or immune systems.

- Less sentient organisms often lack the necessary anatomical and physiological features to model these processes accurately. For example, invertebrates lack an adaptive immune system or the complexity of mammalian neurophysiology.

4. Ethical Considerations and the 3Rs

- The use of sentient animals is a last resort when less sentient organisms cannot yield reliable or relevant results.

- Replacement with alternative methods (e.g., computer simulations, cell cultures) or less sentient species is considered first, but they often fail to replicate the in vivo (inside the body) dynamics necessary for the research.

- By focusing on sentient animals only when required, we adhere to the 3Rs principle of ethical research.

5. Regulatory and Translational Requirements

- In many cases, regulatory agencies require testing on mammalian models to approve new therapies, drugs, or vaccines.

- Data derived from less sentient species are often insufficient for preclinical evaluation due to significant biological differences.

6. Advancing Human and Animal Health

- Studies conducted in mice often have a direct translational impact, contributing to the development of treatments for both humans and animals.

- Using an inappropriate model could lead to inconclusive or invalid results, delaying scientific progress and necessitating the use of more animals overall.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Downloaded: 8:42am, 26 Sep 2025

In this project, we will perform different types of experimental procedures. We refine procedures to minimise harms to animals. The highly trained staff at the animal facility offer significant assistance and ideas for refining protocols and post-procedure checks

1. Use of Anaesthesia and Analgesia

All surgical or invasive procedures will be conducted under appropriate anaesthesia to eliminate pain during the procedure. Post-operative analgesics will be administered to minimize discomfort.

2. Use of Non-Invasive and Minimally Invasive Techniques

- Whenever possible, we will use non-invasive technologies such as imaging to monitor biological processes.

- Blood, tissue, or other sample collection will be minimized by calculating the required volume and using advanced analytical methods to obtain data from smaller samples.

3. Improved Animal Handling

- Animals will be allowed to get used to handling and the research environment to reduce stress during procedures.

- Techniques such as tunnel handling (instead of tail handling for rodents) will be used to reduce stress levels in animals, particularly in aged animals.

4. Housing and Environmental Enrichment

- Housing conditions will meet or exceed regulatory standards, with stable temperature, humidity, light cycles, and noise control. To satisfy the natural social instinct of mice, animals will be housed in compatible groups to reduce stress and isolation, unless the study design requires individual housing or if aggressive behaviour is observed.

- Animals will be provided with enrichment such as nesting material, shelters, and social housing to promote natural behaviours and reduce stress.

5. Skilled Personnel and Continuous Training

All procedures will be conducted efficiently by skilled and well-trained personnel, ensuring they are completed in the shortest time possible to minimize stress and discomfort for the animals. Regular training and updates on best practices for animal care will be conducted.

6. Monitoring and Intervention

- Animals will be closely monitored for any signs of pain, distress, or illness. If such signs are observed, intervention protocols, including administering additional analgesia or implementing humane endpoints, will be promptly applied.

- The well-being of ageing animals will be carefully monitored through regular checks of their condition. Standard checks will include observations of their activity, skin and fur condition, teeth,

Downloaded: 8:42am, 26 Sep 2025

posture and weight. The observations will be cross-checked with previous reports to detect any signs of deterioration. When deterioration is observed, appropriate countermeasures will be taken, which may include supportive diet, additional cage enrichment or specific treatments (e.g. medicaments applied to the skin) upon consultation with the Named Veterinary Surgeon and Named Animal Care and Welfare Officer.

- Detailed welfare scoring systems will be used to objectively assess animal well-being and adjust care or procedures as needed for the studies.

7. Humane Endpoints

Humane endpoints will be clearly defined and strictly adhered to, ensuring unnecessary suffering is avoided. Animals will be humanely killed if predetermined welfare thresholds are met.

8. Refined Experimental Techniques and colony management

- Employing single-cell or high-throughput technologies will reduce the number of animals required while maximizing data output.

- Micro-sampling and refined surgical tools will be employed to reduce procedural invasiveness and recovery time. Ear notching not only provide a method for animal identification but is also used for determine their genetic profiles, all of this information is stored in a well-designed web-based system to enable easy access and efficient management of mouse colonies.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

https://www.nc3rs.org.uk is our reference for best practice in animal research. Various aspects of working with animals such as handling, anaesthesia, analgesia, blood sampling, euthanasia and welfare assessment are covered there under the specific section as in the link:

https://www.nc3rs.org.uk/topic-specific-resources-0

Equally, we will refer to http://www.lasa.co.uk/ where under the tab “Current Publications”, we can find "Guiding Principles" for different aspects of working with animals. Particularly rich information can be found there regarding the principles for aseptic surgery:
http://lasa.co.uk/PDF/LASA_Guiding_Principles_Aseptic_Surgery_2010.2.pdf

To determine the number of animals matching our experimental needs, we will follow the assessment framework available from the Home Office under the link:

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf

For best practice, all experiments will be planned in agreement with PREPARE guidelines (https://norecopa.no/prepare) and we will follow the ARRIVE guidelines (https://norecopa.no/3r-guide/arrive-guidelines) to report our animal research.

Downloaded: 8:42am, 26 Sep 2025

All PIL holders working under this PPL will be strongly encouraged to thoroughly research through and implement these resources in their experiments.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We stay updated with advancements in the 3Rs (Replacement, Reduction, and Refinement) by regularly reviewing newsletters provided by our establishment’s Named Information Officer. We will check the NC3Rs website (https://nc3rs.org.uk/) for updates and follow publications on the LASA forum (https://www.lasa.co.uk/current_publications/). Additionally, we use the establishment's 3Rs Search Tool to explore relevant resources.

All team members are encouraged to familiarize themselves with these materials. Any important updates related to the care and welfare of our mice will be shared during lab meetings or via email lists. We strive to set a good example for our colleagues and maintain a close working relationship with the animal facility staff to ensure the best possible care for the animals under our supervision.

Downloaded: 8:42am, 26 Sep 2025